18B/2000G290, 2002G310

Crystal structure of a novel antifungal peptide distinct with five disulfide bridges from *Ecommia ulmoides* Oliver

Ye Xiang[1], Ren-Huai Huang, Wei Liu[1], Xiao-Zhu Liu[2], Da-Cheng Wang*[1]
[1]Center for Molecular Biology, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, P.R.China
[2]Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P.R.China

Introduction

EAFP2 is a small peptide present in the bark of the tree *Ecommia ulmoides* Oliver (also celled hard-rubber tree or Gutta-percha tree). It is a highly basic protein and able to bind reversibly to chitin, a β (1→4) linked N-acetylglucosamide (GlcNAc) polysaccharide. EAFP2 has been suggested to play a role in plant defence and has shown to possess antifungal activity against several agriculturally important plant pathogenic fungi. The amino acid sequence shows that it consists of 41 residues and distinctly contains ten cysteines cross-linked to form five disulfide bridges with pairing pattern (C1-C5, C2-C9, C3-C6, C4-C7, C8-C10) (Huang *et al*., 2002). This is the first finding of plant antifungal proteins with such a five-disulfide motif. Therefore the three-dimensional structure of EAFP2 becomes most interesting. As the first step, here we report the crystallization, data collection and initial structural analysis of EAFP2, including the *ab initio* structure determination procedure.

Experimental

EAFP2 was isolated from the bark pieces of tree *Eucommia ulmoides* Oliver as described in previous report (Liu *et al*., 1994).

The hang-drop vapor-diffusion method was used for the crystallization of EAFP2. Each drop contained equal amounts (3 µl) of protein (15 mg/ml) and reservoir solutions equilibrated against 500 µl of reservoir solution in the well. Crystals were grown in the following conditions: drops formed by mixing equal volumes (3 µl) of 15 mg/ml protein in water and 1.0 M sodium acetate-acetic acid buffer (pH 5.5) containing 5% (v/v) iso-propanol were equilibrated with the same buffer solution. The crystals appeared in one week and grew to their full size in three weeks. They are typical large thin plate-like with maximum dimensions of 0.6×0.5×0.06 mm and diffract to a very high resolution (Xiang *et al*., 2002).

The X-ray diffraction data collection of EAFP2 at 0.84 Å high resolution were carried out by using synchrotron radiation at BL-18B beamline of the Photon Factory in KEK, Tsukuba, Japan. All data were processed and scaled with the *HKL* suite of programs.

Result and discussion

The structure of EAFP2 was determined *ab initio* by direct methods using *SnB* (Weeks *et al*., 1999) at 0.84 Å resolution and refined with *SHELX97* against experimental intensities to a final R factor of 6.84%.

The general fold of EAFP2 is plate-like. It contains some short secondary structure elements, including two α-helices and a three β-stranded antiparallel β-sheet. Five β-turns are found in connection of these secondary structures and can be classified into three types I, II and VIa. The atomic details of the structure and the possible antifungal mechanism are discussed.

References

[1] R. H. Huang et al., *FEBS Lett*. **521**, 87-90 (2002).

[2] Y. Xiang et al., *Acta Cryst*. **D58**, 1838-1840 (2002).

[3] X. Z. Liu et al., *Acta Bot. Yunnanica* **16**, 385-391 (1994).

[4] C. M. Weeks et al., *J. Appl. Cryst* **32**, 1631-1640 (1999).

* dcwang@sun5.ibp.ac.cn